

## Influence of Agar-Agar Polysaccharide on Changes in Gastrointestinal Protein Hydrolysis

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**Annotation:** Studied the influence of the interaction of indigestible agar-agar polysaccharide and proteins on the change in gastric hydrolysis of proteins. The study was carried out in vitro, in the work used gastric juice, agar-agar, solutions of casein, albumin and hemoglobin. It was concluded that the use of a mixture of agar-agar with proteins helps to reduce protein hydrolysis by gastric juice, due to the formation of agar-agar - protein complexes that prevent protein hydrolysis, and reduce the access of gastric proteases to proteins in the agar-agar-protein complex. An increase in the ratio of agar-agar and protein towards an increase in agar-agar contributes to an additional decrease in protein hydrolysis, which may be an additional decrease in the access of gastric proteases to proteins, in addition to an obstacle to proteins in the agar-agar-protein complex. Thus, protein hydrolysis by gastric juice depends on the interaction agar-agar with proteins as a result of the formation agar-agar-protein complexes, as well as from an increase in the amount agar-agar, which is also an obstacle to the access of gastric proteases to proteins.

**Key words:** In vitro, agar-agar, casein, albumin, hemoglobin, gastric proteases, polysaccharide.

Protein-polysaccharide combinations that lead to the formation of electrostatic complexes and coacervates have been the subject of extensive research using both layered and mixed emulsion approaches. Protein-polysaccharide conjugates have shown interesting physicochemical properties as stabilizers and emulsifiers, as well as food texture modifiers. In addition, they are potential optimal nutrient delivery systems. Their complex behavior due to several factors such as pH, ionic strength, concentration, heating and mechanical processing is the main reason for the constant growth of the research area [4].

Of the polysaccharides, in addition to starch, indigestible polysaccharides used in the food industry are of interest, to improve the texture and stability of food products, which can reduce protein digestibility and therefore alter the bioavailability of amino acids [eight]. Also shown their ability to partially inhibit the activity of proteolytic enzymes in the gastrointestinal tract [5]. Besides, in under simulated

gastric conditions, whey protein and alginate nanoparticles do not digestible polysaccharide in a 2:1 ratio showed the highest protection of whey proteins from pepsin digestion [7].

The activity of trypsin, amylase, lipase was analyzed in human duodenal juice after incubation with various indigestible polysaccharides. The studied polysaccharides reduced the activity of amylase by 35–100%, lipase by 40–95%, and trypsin by 40–85%. It was concluded that various indigestible polysaccharides have the ability to inhibit the activity of pancreatic enzymes. This inhibitory effect depends on the type of polysaccharide and affects different enzymes differently [6].

Of particular interest among the indigestible polysaccharides is agar-agar used as a substitute for gelatin, thickener for soups, fruit preserves, ice cream and other desserts. However, there are very few studies related to agar complexation, and as is known, gelatin-agar systems have been mainly studied [3,ten,nine]. Since gelatin is a structural non-globular protein, unlike, for example, whey proteins. Complexation studies were conducted between whey protein isolate (WPI) and agar, and their interaction was monitored depending on the physico-chemical properties of the agar, pH and ionic strength of the medium. The agars showed excellent properties for complexation with IBP. It was found that the binding of IBP to agar samples is the result of the influence of various factors [eleven].

**Purpose of the study:** explore influence of agar-agar polysaccharide on changes in gastric hydrolysis of proteins.

**Material and methods.** In vitro work studied in the influence of the interaction of agar-agar polysaccharide and casein proteins, egg albumin (albumin) and hemoglobin on the hydrolysis of proteins under the influence of gastric juice. The total proteolytic activity (OPA) of gastric juice was studied[1] using as a substrate both proteins alone and agar-agar and each of the proteins after a preliminary 30-minute joint incubation. Different ratios of agar-agar and protein were used: 1 - only protein, 2 - 1 part of agar-agar and 5 parts of protein, 3 - 1 part of agar-agar and 1 part of protein, 4 - 5 parts of agar-agar and 1 part of protein. Proteolytic activity was studied after 30 and 60 minutes of exposure to gastric juice on a mixture of agar-agar and the protein under study.

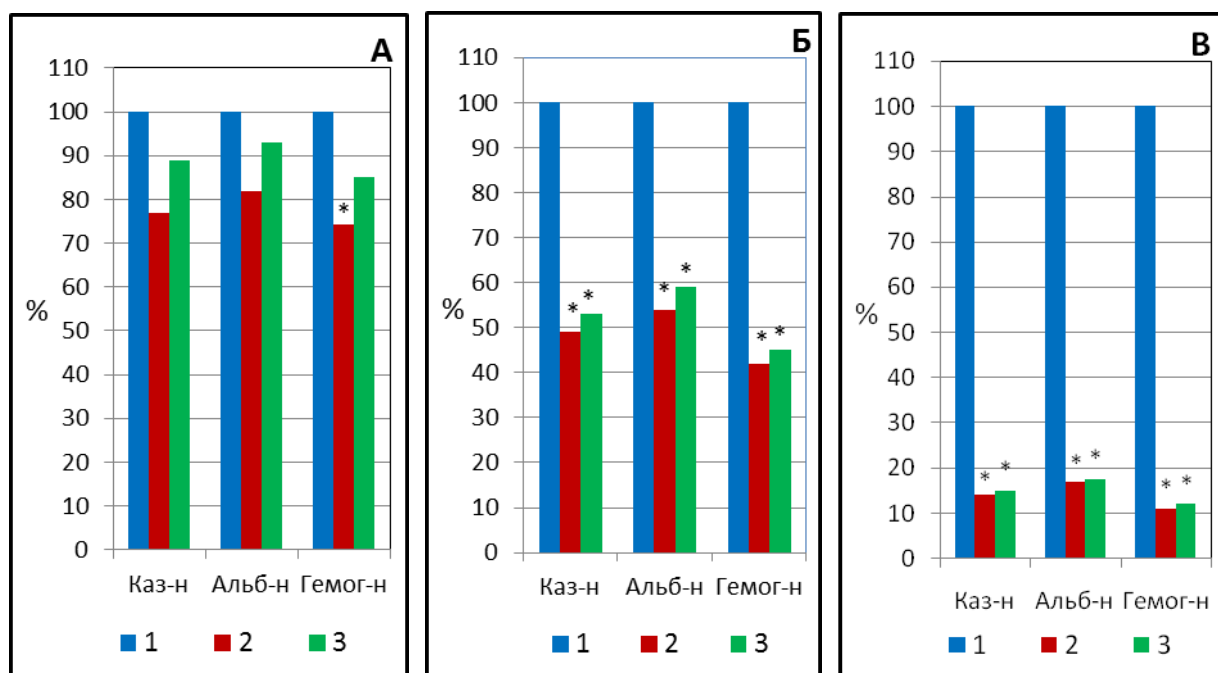
At the same time, the study of changes in the TSA using the studied protein with gastric juice was taken as 100%. Changes in TSA using a mixture of: agar-agar + casein, agar-agar + albumin and agar-agar + hemoglobin, both at 30 min and 60 min of incubation with gastric juice, were calculated in % relative to using only protein.

Statistical processing was carried out by the method of variational statistics with the calculation of average values and their average errors, determination of the coefficient of reliability of the Student-Fisher difference (t). Differences were considered statistically significant at  $p < 0.05$  or less.

**Results.** From the results obtained, it was found that where studied the influence of the interaction of agar-agar and casein on the index of activity of gastric juice. With a ratio of starch and casein of 1:5, it was found that OPA with the use of agar-agar and casein together after 30 minutes of exposure to gastric juice was 77 in relation to the use of casein alone  $\pm 6.9\%$ . This was not significantly lower than that using only case in. At the same time, the application agar-agar and casein after 60 minutes of exposure to gastric juice, the result was  $89 \pm 8.2\%$ , which was also not significantly lower than with casein alone (Fig. A).

When researching the influence of the interaction of agar-agar and albumin on the gastric juice OPA, it was found that with a ratio of agar-agar and albumin 1:5, after 30 minutes of exposure to gastric juice. The OPA indicator was not significantly less similar result using only albumin, and was at the level of  $82 \pm 7.5\%$ . However, when using a mixture agar-agar and albumin after 60 minutes of exposure to gastric juice, the OPA was  $93 \pm 8.6\%$ . At the same time, this result was not significantly lower than a similar indicator using only albumin (Fig. A).

A close focus of the results of the study was established on gastric juice OPA and under interaction influence agar- agar.



**Picture.** Investigation of changes in gastric juice OPA when used as a substrate proteins, as well as mixtures agar-agar with proteins casein, albumin and hemoglobin in the ratio agar-agar and protein: A - 1:5, B - 1:1, C - 5:1. 1 - OPA when using casein, albumin and hemoglobin with gastric juice taken as 100%. 2 - OPA in% when using a mixture: agar-agar+ casein, agar-agar +albumin and agar-agar+ hemoglobin, with 30 min of incubation with gastric juice. 3 - OPA in% when using a mixture: agar-agar+ casein, agar-agar +albumin and agar-agar+ hemoglobin, at 60 min of incubation with gastric juice.

➤ Significantly different values of the change in the OPA in relation to the use of only protein as a substrate.

Agar and hemoglobin at a ratio of 1:5. At the same time, the OPA index after 30 min of exposure to gastric juice was significantly less similar result using only hemoglobin and was equal to  $74 \pm 6.9\%$ . At the same time, the use of a mixture agar-agar and hemoglobin after 60 minutes of exposure to gastric juice, this indicator corresponded to the level of  $85 \pm 7.9\%$ , which was not significantly less than the same result using only hemoglobin (Fig. A).

When researching interaction of agar-agar and casein in a ratio of 1:1, OPA after 30 minutes of exposure to gastric juice was  $49 \pm 4.2\%$ , this indicator was significantly and significantly lower than the similar result using only casein, it was also significantly lower than the similar result using agar-agar and casein in the ratio 1:5. At the same time, the use agar-agar and casein after 60 minutes of exposure to gastric juice, the OPA was  $53 \pm 4.7\%$ , which was significantly lower than a similar result using only casein, and also significantly lower than a similar result of the mixture agar-agar and casein with a ratio 1:5 (Fig. B).

Also it was found that with the use of agar-agar and albumin in a ratio of 1:1, after 30 minutes of exposure to gastric juice, the OPA was significantly lower similar result with albumin alone and was  $54 \pm 4.7\%$ . This result was significantly lower than the similar result using only albumin, and this indicator was not significantly lower than the similar result using combined agar-agar and albumin in the ratio 1:5. Besides mixture use agar-agar and albumin after 60 minutes of exposure to gastric juice,

the OPA value was  $59 \pm 5.1\%$ , which was significantly less than a similar result using only albumin, and also significantly lower than a similar result of the mixture agar-agar and albumin in the ratio 1:5 (Fig. B).

From the data obtained, it was found that when using a mixture of agar-agar and hemoglobin in a ratio of 1: 1, after 30 minutes of exposure to gastric juice OPA was significantly less same indicator using only hemoglobin and was  $42 \pm 3.6\%$ . Also, this value was not significantly lower than the similar result with the use of agar-agar and hemoglobin in relation to 1:5. At the same time, using a mixture agar-agar and hemoglobin after 60 minutes of exposure to gastric juice, the level of OPA was  $45 \pm 3.7\%$ , this result was significantly lower than the similar indicator using only hemoglobin, in addition, significantly less similar result of the mixture agar-agar and hemoglobin in relation to 1:5 (Fig. B).

The results of the study the influence of a mixture of agar-agar and casein in a ratio of 5: 1, after 30 minutes of exposure to gastric juice, the OPA was equal to  $14 \pm 1.1\%$ , this indicator was significantly and significantly lower than the similar result with the use of casein alone. In addition, this indicator was significantly lower than a similar result using a mixture agar-agar and casein with a ratio like 1:5 and 1:1. However, the use of a mixture agar-agar and casein after 60 minutes of exposure to gastric juice, the OPA was  $15 \pm 1.2\%$ , which was significantly lower than the similar result using only casein, and also significantly lower than the similar result of the mixture agar-agar and casein with a ratio 1:5 and 1:1 (Fig. B).

In addition to this, it was established that the combined use of agar-agar and albumin in a ratio of 5:1, after 30 minutes of exposure to gastric juice, caused a change in the OPA, which was significantly lower similar result using only albumin and was  $17 \pm 1.4\%$ . This result was significantly lower than the similar value with the use of albumin alone, and this indicator is not significantly lower than the similar result with the use of combined agar-agar and albumin in relation to 1:5 and 1:1. Where in using a mixture agar-agar and albumin after 60 minutes of exposure to gastric juice, the OPA was  $17.6 \pm 1.5\%$ , which was significantly lower than the similar indicator using only albumin, and also significantly lower than the similar result of the mixture agar-agar and albumin in relation to 1:5, as well as 1:1 (Fig. B).

At the same time, it was found that when using a mixture of agar-agar and hemoglobin in a ratio of 5: 1, after 30 minutes of exposure to gastric juice OPA was significantly lower a similar indicator of only hemoglobin and amounted to  $11 \pm 0.8\%$ . Besides OPA was significantly less than the same result using a mixture agar-agar and hemoglobin in relation to 1:5, and 1:1. However, using a mixture agar-agar and hemoglobin after 60 minutes of exposure to gastric juice, the OPA was equal to  $12 \pm 0.9\%$ , which was significantly lower than a similar result with only hemoglobin and significantly lower than the same indicator of the mixture agar-agar and hemoglobin in relation to 1:5 and 1:1 (Fig. B).

**The discussion of the results.** The results of these studies showed that OPA depends on the interaction agar-agar with proteins through the formation agar-agar-protein complexes. This is confirmed by the data we obtained, where it was found that with use as a substrate of agar-agar together with casein in the ratio 1:5 and 30 min of pre-incubation of this mixture, as well as a further 30 min exposure to the mixture of gastric juice. OPA was not significantly lower than when using only casein as a substrate under similar conditions. At the same time, when the gastric juice mixture was exposed to gastric juice for 60 minutes, the OPA increased relative to 30 minutes of incubation, but was not significantly lower compared to using only casein as a substrate. A similar direction of changes, under the same conditions, but with greater values of OPA, was also noted when using as a substrate for agar-agar together with albumin. And using agar-agar together with hemoglobin was significantly lower at 30 min of incubation and not significantly lower at 60 min of incubation in relation to using only hemoglobin as a substrate.

At use as a substrate of agar-agar together with casein in the ratio 1:1 and also similar conditions of incubation of the mixture without gastric juice and further with 30 min exposure to gastric juice. There were more pronounced changes associated with a decrease in OPA than with the ratio 1:5, and these results were significantly lower than when using only casein as a substrate under similar conditions. At the same time, 60 min exposure to the mixture agar-agar with casein of gastric juice caused a slight increase in OPA compared with 30 min exposure, at the same time, this figure was significantly lower compared to the result of using only casein as a substrate. Similar changes in the OPA, under the same conditions, were observed when using as a substrate for agar-agar together with albumin in the ratio 1:1, this indicator was significantly less than in the ratio 1:5. At the same time, significantly lower OPA values were noted both at 30 and 60 minutes of exposure to gastric juice, compared with the results of using only albumin. However, with a slight increase at 60 min exposure relative to 30 min. The same changes were noted when using agar-agar together with hemoglobin in the ratio 1:1, but with lower rates. Also smaller values than in relation to 1:5.

Using a mixture agar-agar and casein in the ratio 5:1, with similar conditions for incubation of the mixture without gastric juice and with gastric juice. The direction of the change in the OPA was noted, as with the ratio 1:1, and 1:5, but with the smallest values than with these ratios. At the same time, there were significantly and significantly lower OPA values both at 30 and 60 min exposure to gastric juice using agar-agar together with each protein casein, albumin, hemoglobin compared to the values separately for each protein. Also revealed the most minimal increase in the parameters of the OPA at 60 min of incubation in relation to 30 min of incubation.

The obtained results of the study show that the use of a mixture of agar-agar with proteins helps to reduce protein hydrolysis by gastric juice, especially with an increase in its concentration relative to protein. This may be due to the formation of agar-agar-protein complexes that prevent the hydrolysis of proteins, by reducing the access of gastric proteases to proteins in the agar-agar-protein complex. With an increase in the ratio of agar-agar and protein in the direction of increasing agar-agar, it contributes to an additional decrease in protein hydrolysis, which may be due to the fact that an increase in the amount of agar-agar may be an additional decrease in the access of gastric proteases to proteins, in addition to an obstacle to proteins in agar-agar-protein complex. Thus, protein hydrolysis by gastric juice depends on the interaction agar-agar with proteins as a result of the formation of starch-protein complexes, as well as on the amount agar-agar, an increase in the concentration of which also contributes to an obstacle to the access of gastric proteases to proteins.

**Findings:** The use of a mixture of agar-agar with proteins helps to reduce protein hydrolysis by gastric juice, due to the formation of agar-agar - protein complexes that prevent protein hydrolysis, and reduce the access of gastric proteases to proteins in the agar-agar-protein complex. An increase in the ratio of agar-agar and protein towards an increase in agar-agar contributes to an additional decrease in protein hydrolysis, which may be an additional decrease in the access of gastric proteases to proteins, in addition to an obstacle to proteins in the agar-agar-protein complex. Thus, protein hydrolysis by gastric juice depends on the interaction agar-agar with proteins as a result of the formation agar-agar-protein complexes, as well as from an increase in the amount agar-agar, which is also an obstacle to the access of gastric proteases to proteins.

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